UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/758,401	01/15/2004	G. Mike Makrigiorgos	700157-53471	6102

7590 09/18/2007

Ronald I. Eisenstein
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110

EXAMINER	
BAUGHMAN, MOLLY E	
ART UNIT	**PAPER NUMBER**
1637	

MAIL DATE	DELIVERY MODE
09/18/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/758,401	MAKRIGIORGOS, G. MIKE	
	Examiner	Art Unit	
	Molly E. Baughman	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *22 June 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2,6-12,15-17 and 35-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2,6-12,15-17 and 35-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendments to claims 2, 6, and 8; cancellation of claims 11, and 13-14; and addition of claims 35-37 in the reply filed 6/22/2007 are acknowledged.

2. Applicant's arguments, see pg.11-13, filed 6/22/2007, with respect to the rejection(s) of claim(s) 2 and 6-17 under 35 USC § 112 have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made over claims 6, 15, and 35.

3. Applicant's arguments, see pg.13-14, filed 6/22/2007, with respect to the rejection(s) of claim(s) 2 under 35 USC § 102 (Weissman, US 6,235,502) have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Weissman (US 6,235,502) and Kaur et al., "Ligation of a primer at a mutation: a method to detect low level mutations in DNA," Mutagenesis, 2002, Vol.17, No.5, pp.365-373 (of record).

4. Applicant's arguments, see pg.14-16, filed 6/22/2007, with respect to the rejection(s) of claim(s) 2 under 35 USC § 103 (Ahern in view of Liu et al.) have been fully considered and are persuasive in view of the amendments. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Ahern and Kaur et al. (Mutagenesis, 2002).

5. Applicant's arguments, see pg.17, filed 6/22/2007, with respect to the rejection(s) of claim(s) 2 under 35 USC § 103 (Abarazua in view of Liu et al.) have been fully considered and are persuasive in view of the amendments. Therefore, the rejection has

been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Abarazua and Kaur et al. (Mutagenesis, 2002).

6. Applicant's arguments, see pg.16, filed 6/22/2007, with respect to the rejection(s) of claim(s) 6-17 under 35 USC § 103 (Weissman in view of Wagner, US 6,114,115) have been fully considered and are persuasive in view of the amendments. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Weissman, Kaur et al., and Wagner.

7. Applicant's arguments, see pg.16-17, filed 6/22/2007, with respect to the rejection(s) of claim(s) 6-17 under 35 USC § 103 (Ahern in view of Wagner, US 6,114,115) have been fully considered and are persuasive in view of the amendments. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Ahern, Kaur et al., and Wagner.

8. Applicant's arguments, see pg.17-18, filed 6/22/2007, with respect to the rejection(s) of claim(s) 6-17 under 35 USC § 103 (Abarzua in view of Wagner, US 6,114,115) have been fully considered and are persuasive in view of the amendments. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Abarzua, Kaur et al., and Wagner.

Claim Rejections - 35 USC § 112

9. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 6-12, 15-17, and 35-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claims 6-12, 15-17, and 36-37 are confusing because it cannot be determined what is encompassed by "converting the linear double stranded PCR products into a plurality of second hairpin structures comprising a double-stranded complementary region and a non-complementary single-stranded loop at the end of the double-stranded region by a method which induces denaturation of the linear double stranded PCR products followed by sudden renaturation wherein the plurality of the second hairpin structures are formed by hybridization of the amplified sequence of interest and its complementary sequence that are present in each denaturated single stranded PCR products flanked by the first and second single stranded non-complementary single stranded 5' and 3' nucleic acid sequences" in claim 6. While the breath of the step is understood, the sentence as written is grammatically confusing and renders the step unclear. The claim language describing the second hairpin structures (...comprising a double-stranded complementary region...) is unnecessary and renders the claim confusing. The claim language describing how the hairpin structures are formed (...hybridization of the amplified sequence...) is confusing because each single stranded PCR product contains an amplified sequence of interest and its complementary sequence, and therefore could hyrbidize with each other. It is suggested to amend step (b) in the claim to further describe what each strand

within the double stranded PCR product now consists of, and provide a further description of how each of the strands within the double-stranded PCR product hybridize during renaturation in step (c). For instance, step (b) could entail further description of the double stranded PCR products by: "...double stranded PCR products wherein each strand of the linear double stranded PCR products comprises an amplified sequence of interest, its complement, a cap sequence between the amplified sequence of interest and its complement, flanked 5' and 3' by the first and second single stranded non-complementary single stranded nucleic acid sequences." Step (c) could entail a further description of the renaturation by: "converting the linear double stranded PCR products into a plurality of second hairpin structures by a method which induces denaturation of the linear double stranded PCR products into single stranded PCR products, followed by sudden renaturation, wherein the amplified sequence of interest and its complement within each single strand hybridize during renaturation, thereby forming a hairpin structure."

b. Claims 15-17 are confusing because claim 15 does not recite any active steps. For example, the method is drawn to an assay which relies on a generated template from the method of claim 6, however, the method does not comprise any steps of the method using such a generated template. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986).

c. Claim 35 is confusing because it recites ligating a cap containing a sequence about midway in the cap unamplifiable by PCR, wherein the nucleic acid bases on either side of this sequence are not complementary to each other, however the specification also states that both caps naturally form hairpins on their own (pg.14), and therefore, must comprise sequences complementary to each other as well. The sequences on either side of the unamplifiable region can only comprise sequences which are non-complementary to each other. Correction is required.

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Weissman (US 6,235,502, of record) in view of Kaur et al., "Ligation of a primer at a mutation: a method to detect low level mutations in DNA," Mutagenesis, 2002, Vol.17, No.5, pp.365-373 (of record).

Weissmen et al. describe a method of ligating hairpin forming adaptors to the ends of DNA fragments for rolling circle amplification, where the hairpins have 3' and.5' ends that are complementary to each other and form stem and loop structures, thereby forming a hairpin structure with the target (abstract, and column 4, lines 57-62).

Adaptors can also be linear or Y shaped (Figure 1 A, column 3, lines 22-26, column 6, lines 42-63). In Figure 2, Weissmen discusses an embodiment wherein two adaptor comprising a "cap" are ligated to the ends of the double-stranded DNA fragment and a primer to a sequence within the loop of the hairpin adaptor is be used for PCR (Figure 2, column 5, lines 1-3, column 9, lines 8-10). Weissmen also discusses the use of abasic sites, hairpin structures, or protein binding sites on the adaptor sequences that either slow, partially obstruct, or completely block a DNA polymerase (column 3-4 and column 7-8).

Although Weissman discusses the method wherein both strands are amplified using a primer to the adaptor sequence (Fig.2), he does not discuss the method wherein the Y shaped adaptor or two single stranded sequences ligated to one end are used as priming sites for PCR in the same reaction.

Kaur et al. describe a method comprising ligating two non-complementary single-stranded sequences to the 5' and 3' end of a double-stranded DNA fragment, wherein these "linkers" are used as priming sites for subsequent amplification (See abstract, Fig.1, pp.366, "Dephosphorylation and ligation," and "The first and second rounds of PCR").

One of ordinary skill in the art would have been motivated to modify the method of Weissman et al. to ligate two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid for use as priming sites in subsequent PCR because Kaur et al. demonstrate that ligation of two non-complementary nucleic acids for use in subsequent PCR amplification was conventional in the art at the time of

the invention, and further states that "the approach is easy to adopt, since the steps involved (digestion, ligation, dephosphorylation, and amplification) are common practice in most laboratories" (pg.371, 1st column). Furthermore, amplification using a primer targeted to a hairpin adaptor, or "cap," (as in Weissman) and primers targeting two non-complementary single stranded adaptors (as in Kaur) both result in amplification of both strands of a double-stranded template sequence creating a product comprising both the sequence of interest, its complement, and a "cap" there between, and could therefore, easily be substituted for one another. Therefore, the skilled artisan would have had a reasonable expectation of success in ligating two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid instead of a "cap," for use as priming sites in subsequent PCR in the method of Weissman et al. It would have been obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed two non-complementary single-stranded sequences therein.

13. Claims 6-12, 15-17, and 36-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Weissman in view of Kaur as applied to claim 2 above, and further in view of Wagner (U.S. 6,114,115).

The teachings of the primary references are discussed above. Although Weissman also discusses using a polymerase that has 3' →5' exonuclease activity capable of removing misincorporated nucleotides, thereby correcting base mispairs (column 3, lines 44-45; column 9, lines 16-20), he does not particularly discuss

converting the PCR products into hairpin structures by a method which induces denaturation followed by sudden renaturation, identifying hairpins containing mismatches or mutations, removing them, and collecting the DNA containing no mismatches [claims 6-12]. These references also do not discuss a method wherein the concentration of primers are either equal to each other or unbalanced [claim 36-37]. They do not teach using the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection, mutation analysis, polymorphism detection, polymorphism analysis, microsatellite analysis, or cloning and protein functional analysis, wherein such mutation or polymorphism detection methods are selected from the group consisting of PCR, PCR/RE/LCR, MutEx-ACB-PCR, RFLP analysis, APRIL-ATM [claims 15-17].

Wagner describes a method using an immobilized DNA mismatch-binding protein, such as MutS, to isolate or remove duplex DNA molecules containing mismatches such as error-containing molecules in PCR-amplified DNA samples (abstract). The method comprises (a) subjecting amplified DNA to conditions of denaturation followed by reannealing, such that the error-containing or the minority sequences form heteroduplexes containing a mismatch, thereby generating a mixture of perfectly matched duplexes and the heteroduplexes, (b) including the mixture with an immobilized mismatch binding protein wherein the heteroduplexes bind to the protein, and (c) removing the immobilized protein, thereby removing the majority of the sequences containing sequence errors from the amplified DNA sample (column 9, lines 12-35). In part (b) of the method, Wagner also further explains that the denatured/

reannealed DNA is in a state wherein incorrect bases are found in mismatched base pairs after annealing [i.e. conditions which create hairpin structures] (column 27, lines 10-13). In his method, the material which does not bind to the mismatch binding proteins (MBP) is recovered, and contains only those duplex sequences without mismatches (column 6, lines 48-53). In one embodiment, Wagner also describes template strands which comprise trinucleotide repeats, wherein a secondary structure, in particular, a hairpin structure with complementary heteroduplexes, can bind to immobilized MBPs such as MutS after denaturation / renaturation (column 31-32, and column 49, lines 3-12). Denaturing, followed by reannealing for homozygous and heterozygous DNA occurs under conditions which comprise treating the amplified products to 100°C for 4 minutes, followed by 50°C for 60 minutes (column 46, lines 65-67). Conditions for DNA comprising triplet repeat sequences comprise heating the DNA to 70°C for 10 minutes, followed by cooling for 45 minutes at room temperature, and then cooled further at 4°C (column 48, lines 45-47). In several experiments, Wagner discusses PCR amplification using primers at unequal concentrations, 0.1uM primer #1, 0.075 uM primer #2 (column 41, lines 26-27), as well as at equal concentrations, 0.2 uM primer#1, 0.2 uM primer#2 (column 41, lines 65-66). Wagner describes using the method to eliminate all DNA molecules with sequence alterations introduced by PCR copy errors for accurate mutation detection, or reducing the risk of PCR amplifying a nucleotide sequence different from the starting sequence in cloning experiments (column 26, lines 40-54).

One of ordinary skill in the art would have been motivated to modify the method of Weissman and Kaur to use MutS to remove mismatch duplex DNA molecules, to use primers at equal or unequal concentrations, and use the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection via PCR because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37). Wagner also demonstrates the benefits of using primers at equal or unequal concentrations in his examples, as well as using the method to ensure for accurate mutation detection or cloning via PCR. Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides, using primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning in the method of Weissman and Kaur. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules, primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning therein.

14. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ahern (U.S. 5,470,724, of record) in view of Kaur et al., "Ligation of a primer at a mutation: a method to detect low level mutations in DNA," Mutagenesis, 2002, Vol.17, No.5, pp.365-373 (of record).

Ahern describes a method of amplifying DNA sequences of interest by cleaving DNA to produce discrete fragments, ligating the fragments to adaptor polynucleotides having a ligatable end, and first and second self complementary sequences separated by a spacer sequence, thereby forming ligated duplexes and amplifying the ligated duplexes with a polymerase (abstract, Figures 2B, 3A-4, column 2, lines 41-50). The adaptor polynucleotides are called "panhandled" and comprises of ends with complementary sequences and a spacer region inbetween that forms a loop (column 5, lines 8-14) and such an amplification is called "Boomerang DNA Amplification" or "BDA" (entire document). Primers anneal to the primer target sites on the BDA templates (i.e. on the adaptor sequence) and are extended using a polymerizing agent (col.2, lines 55-67; column 3, lines 8-10; Fig.3C and 4).

Although Ahern discusses the method wherein both strands are amplified using primers to one of the adaptor sequences (Fig.3C and 4), he does not discuss the method wherein two single stranded sequences are ligated to one end and are used as priming sites for PCR in the same reaction.

Kaur et al. describe a method comprising ligating two non-complementary single-stranded sequences to the 5' and 3' end of a double-stranded DNA fragment, wherein these "linkers" are used as priming sites for subsequent amplification (See abstract,

Fig.1, pp.366, "Dephosphorylation and ligation," and "The first and second rounds of PCR").

One of ordinary skill in the art would have been motivated to modify the method of Ahern to ligate two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid for use as priming sites in subsequent PCR because Kaur et al. demonstrate that ligation of two non-complementary nucleic acids for use in subsequent PCR amplification was conventional in the art at the time of the invention, and further states that "the approach is easy to adopt, since the steps involved (digestion, ligation, dephosphorylation, and amplification) are common practice in most laboratories" (pg.371, 1st column). Furthermore, amplification using primers targeted to a hairpin adaptor, or "cap," (as in Ahern) and primers targeting two non-complementary single stranded adaptors (as in Kaur) both result in amplification of both strands of a double-stranded template sequence creating a product comprising both the sequence of interest, its complement, and a "cap" there between, and could therefore, easily be substituted for one another. Therefore, the skilled artisan would have had a reasonable expectation of success in ligating two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid instead of a "cap," for use as priming sites in subsequent PCR in the method of Ahern. It would have been obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed two non-complementary single-stranded sequences therein.

15. Claims 6-12, 15-17, and 36-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ahern in view of Kaur as applied to claim 2 above, and further in view of Wagner (U.S. 6,114,115).

The teachings of the primary references are discussed above. Ahern also discusses using the method for a particular application (i.e. further characterizing the PCR amplified products), such as cleaving the amplified DNA using one or more restriction nucleases and subjecting them to restriction mapping analysis on gels (i.e. RFLP) (column 12, lines 11-14, and column 27, lines 10-65), and ligating the amplified sequence of interest into a cloning vector for sequence analysis (column 12, lines 17-20, and columns 29-31). Ahern also discusses using unequal concentrations of primers during BDA and PCR reactions, specifically 2 ug of 21-base primer and 2.7 ug of 30-base primer (column 23, lines 30-31).

These references do not discuss converting the PCR products into hairpin structures by a method which induces denaturation followed by sudden renaturation, identifying hairpins containing mismatches or mutations, removing them, and collecting the DNA containing no mismatches [claims 6-12].

The teachings of Wagner are discussed above, wherein Wagner teaches subjecting amplified PCR products to denaturation, followed by sudden renaturation (i.e. thereby creating hairpin duplex structures), and then using an enzyme (MutS) to identify and remove the sequence structures containing mismatches or mutations, thereby collecting DNA containing no mismatches.

One of ordinary skill in the art would have been motivated to modify the method of Ahern and Kaur to use MutS to remove mismatch duplex DNA molecules prior to a method, such as RFLP or cloning because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37), which can ensure for accurate mutation detection or cloning via PCR. Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides prior to RFLP or cloning analysis in the method of Ahern and Kaur. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules therein.

16. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Abarzua (US 2003/0108902 A1, of record) in view of Kaur et al., "Ligation of a primer at a mutation: a method to detect low level mutations in DNA," Mutagenesis, 2002, Vol.17, No.5, pp.365-373 (of record).

Abarzua discusses a method comprising ligating hairpin oligonucleotides to a restriction enzyme fragment, or non-circular duplex DNA with strands W and C and having a single-stranded overhang at each end such that after hybridization with hairpin oligonucleotides, and subsequent ligation, the ligated product is then amplified via

rolling circle amplification (RCA). Following RCA, the products can be duplexed by self-annealing, and then cut by the corresponding restriction nuclease(s) to generate the original fragment (page 2, paragraph [0024], and Figure 4). Such hairpin oligonucleotides may be designed to incorporate a binding site for universal primer sequences useful in RCA (page 8, paragraph [0070] and page 10, claims 19-20). Abarzua describes subjecting the products to 100°C (heat denaturation), followed by quick cooling to 4°C in water/ice bath, following the reactions (page 8, paragraph [0075]).

Although Abarzua discusses the method wherein both strands are amplified using a primer to an adaptor sequence (page 8, paragraph [0070] and page 10, claims 19-20), he does not discuss the method wherein two single stranded sequences are ligated to one end and are used as priming sites for PCR in the same reaction.

Kaur et al. describe a method comprising ligating two non-complementary single-stranded sequences to the 5' and 3' end of a double-stranded DNA fragment, wherein these "linkers" are used as priming sites for subsequent amplification (See abstract, Fig.1, pp.366, "Dephosphorylation and ligation," and "The first and second rounds of PCR").

One of ordinary skill in the art would have been motivated to modify the method of Abarzua to ligate two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid for use as priming sites in subsequent PCR because Kaur et al. demonstrate that ligation of two non-complementary nucleic acids for use in subsequent PCR amplification was conventional in the art at the time of the

invention, and further states that "the approach is easy to adopt, since the steps involved (digestion, ligation, dephosphorylation, and amplification) are common practice in most laboratories" (pg.371, 1st column). Furthermore, amplification using a primer targeted to a hairpin adaptor, or "cap," (as in Abarzua) and primers targeting two non-complementary single stranded adaptors (as in Kaur) both result in amplification of both strands of a double-stranded template sequence creating a product comprising both the sequence of interest, its complement, and a "cap" there between, and could therefore, easily be substituted for one another. Therefore, the skilled artisan would have had a reasonable expectation of success in ligating two non-complementary single-stranded sequences to one end of the double-stranded template nucleic acid instead of a "cap," for use as priming sites in subsequent PCR in the method of Abarzua. It would have been obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed two non-complementary single-stranded sequences therein.

17. Claims 6-12, 15-17, and 36-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Abarzua in view of Kaur as applied to claim 2 above, and further in view of Wagner (U.S. 6,114,115).

The teachings of the primary references are discussed above. These references do not discuss identifying hairpins containing mismatches or mutations, removing them, and collecting the DNA containing no mismatches [claims 6-12]. They do not discuss a method wherein the concentration of primers are either equal to each other or

unbalanced [claim 36-37]. They do not teach using the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection, mutation analysis, polymorphism detection, polymorphism analysis, microsatellite analysis, or cloning and protein functional analysis, wherein such mutation or polymorphism detection methods are selected from the group consisting of PCR, PCR/RE/LCR, MutEx-ACB-PCR, RFLP analysis, APRIL-ATM [claims 15-17].

The teachings of Wagner are discussed above, wherein Wagner teaches subjecting amplified PCR products to denaturation, followed by sudden renaturation (i.e. thereby creating hairpin duplex structures), and then using an enzyme (MutS) to identify and remove the sequence structures containing mismatches or mutations, thereby collecting DNA containing no mismatches. Wagner discusses using primers at unequal and equal concentrations, as well as using the method to eliminate all DNA molecules with sequence alterations introduced by PCR copy errors for accurate mutation detection, or reducing the risk of PCR amplifying a nucleotide sequence different from the starting sequence in cloning experiments (also discussed above).

One of ordinary skill in the art would have been motivated to modify the method of Abarzua and Kaur to use MutS to remove duplexed self-annealed products that contain mismatches or mutations, to use primers at equal or unequal concentrations, and use the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection via PCR because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an

immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37). Wagner also demonstrates the benefits of using primers at equal or unequal concentrations in his examples, as well as using the method to ensure for accurate mutation detection or cloning via PCR. Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides, using primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning in the method of Abarzua and Kaur. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules, primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning therein.

18. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Weissman et al. (US 6,235,502) in view of Ahern (5,470,724).

The teachings of Weissman are discussed above. Briefly, Weissman discusses the method wherein two hairpin adaptors (i.e. caps) are ligated to a double-stranded template fragment (Fig.2; col.4, lines 57-67), wherein one of the ligated adaptors can contain one or more abasic sites which pause or completely block the activity of DNA polymerase (col.3-4, and col.7-8). The caps provide a single-stranded region to where a primer can be annealed for PCR (col.5, lines 1-3).

Weissman does not discuss the method wherein two primers are used in the reaction, and wherein each primer binds to at least a portion of the cap on either side of the one or more abasic sites.

As discussed above, Ahern discusses a similar method, where the doubles stranded template is ligated to adaptor polynucleotides having a ligatable end, and first and second self complementary sequences separated by a spacer sequence (i.e. caps), thereby forming ligated duplexes and amplifying the ligated duplexes with a polymerase (abstract, Figures 2B, 3A-4, column 2, lines 41-50). Primers anneal to the primer target sites on the BDA templates (i.e. on the adaptor sequence) and are extended using a polymerizing agent (col.2, lines 55-67; column 3, lines 8-10; Fig.3C and 4).

It is noted that Ahern does not specifically discuss the method wherein the primers anneal to non-complementary sequences on the single stranded cap, but instead demonstrates that it was conventional in the art at the time of the invention to amplify the same structure using two primers which hybridize to different regions of one of the ligated adaptors.

One of ordinary skill in the art would have been motivated to modify the method of Weissman et al. to use two primers during PCR, wherein each primer binds to a non-complementary region on the cap on either side of the one or more abasic sites because Weissman demonstrates that it was conventional in the art to amplify a created hairpin structure using a primer targeted to the ligated cap, and further demonstrates that it was also conventional in the art to insert sequences into the template by ligation which cannot be amplified by PCR, and Ahern demonstrates that it was also

conventional to amplify the same structure using two primers which hybridize to the ligated adaptor. Since the method of Weissman, using a single primer and abasic sites, and the method of Ahern, using two primers directed to different sequences on a ligated adaptor, both result in amplification of both strands of a double-stranded template sequence creating a product comprising both the sequence of interest, its complement, and a "cap" there between, a method using two primers targeting either side of the abasic site(s) could therefore, easily be substituted for one which only uses one primer. Therefore, the skilled artisan would have had a reasonable expectation of success in using two primers during PCR, wherein each primer binds to a non-complementary region on the cap on either side of the one or more abasic sites in the method of Weissman et al. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed two primers therein.

SUMMARY

1. No claims are free of the prior art.

2. Chen et al. (US 7,208,278) is noted as a reference of interest. The recent patent contains subject matter very similar to the instant application, although it cannot be used as prior art due to a later filing date.

CONCLUSIONS

3. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Molly E. Baughman whose telephone number is 571-272-4434. The examiner can normally be reached on Monday-Friday 8-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Molly E Baughman
Examiner
Art Unit 1637

MEB 9/14/07

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

9/17/07